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Application of: Shoemaker et al.

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For: COMPOSITIONS AND METHODS
FOR EXON PROFILING

Attorney Docket No: 9301-123

RECEIVED

**RESPONSE TO RESTRICTION REQUIREMENT AND PRELIMINARY
AMENDMENT UNDER 37 C.F.R. 1.115**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Office Action mailed November 9, 2001 in connection with the above-identified patent application and in accordance with Rule 115 of the Rules of Practice, please enter the following amendments and consider the following remarks. Applicants submit herewith: 1) Exhibit A: marked version of the paragraphs in the specification which have been amended; 2) Exhibit B: clean version of the paragraphs in the specification, as amended; 3) Exhibit C: marked version of the amended claims showing changes made; 4) Exhibit D: clean version of the pending claims, as amended; 5) Petition for Extension of Time for a period of one month from December 9, 2001 to and including January 9, 2002, accompanied by the appropriate fee; 6) Amendment Fee Transmittal, accompanied by the appropriate fee; and 7) Information Disclosure Statement, including revised Form PTO-1449: "List of References Cited by Applicant" and copies of references AA-KD, accompanied by the appropriate fee.

IN THE SPECIFICATION:

A marked version of the following amended paragraphs is attached hereto as Exhibit A. Matter that has been deleted from the paragraph is indicated by brackets and matter that has been added to the paragraph is indicated by underlining. A clean version of the following amended paragraphs is attached hereto as Exhibit B.

Please amend the specification as follows:

On page 4, please replace the paragraph beginning "However, current DNA array technologies typically monitor the 3' ends" with the following paragraph:

However, current DNA array technologies typically monitor the 3' ends of mRNA molecules in a cell, rather than the expression levels of individual exons that make up the mRNAs. For example, probes used in cDNA arrays typically range in sizes from about 0.6 to 2.4kb (Duggan et al., *Nature Genetics Supplement* 21:10-14), and are generally complementary to the 3' ends of the mRNA molecules. Probes used in cDNA arrays are biased to the 3' end because labeling methods typically rely on d(T) primed reverse transcription. Expression analysis using high density oligonucleotide arrays has been described that requires scoring and averaging of as many as 20 oligonucleotide probes on an array, chosen from various locations of the coding sequence of a gene, to determine the transcript level of the corresponding mRNA (see, e.g., Lockhart et al., 1996, *Nature Biotechnology* 14:1675; U.S. Patent Nos. 5,578,832; 5,556,752; 5,510,270; and 6,040,138; Lipshutz et al., 1999, *Nature Genetics Supplement* 21:20-24). Again, these probes are placed near the 3' ends of mRNA molecules and the probe intensities are averaged to a single value, and thus does not provide information of the expression of individual exons across the genes. In addition, it has been found that the majority of splicing events occurs in 5' untranslated regions, which leads to the generation of additional protein domains rather than alternating domains (Mironov et al., 1999, *Genome Research* 9:1288-1293). It has also been found that alternative exon-intron structures, i.e., with different end points, exist in many exons, which leads to expressed exons of different lengths (Mironov et al., 1999, *Genome Research* 9:1288-1293). Thus, there exists a need to design DNA arrays that measure the expression levels and the lengths of a plurality of exons for each of a plurality of genes in the genome of an organism. There exists a need for methods for quantitatively monitoring alternative splicing on a genome-wide scale.

On page 46, please replace the paragraph beginning "In preferred embodiments" with the following paragraph:

In preferred embodiments, the target polynucleotides to be analyzed are prepared *in vitro* from nucleic acids extracted from cells. For example, in one embodiment, RNA is extracted from cells (e.g., total cellular RNA, poly(A)⁺ messenger RNA, fraction thereof) and

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cont

messenger RNA is purified from the total extracted RNA. Methods for preparing total and poly(A)⁺ RNA are well known in the art, and are described generally, e.g., in Sambrook *et al.*, *supra*. In one embodiment, RNA is extracted from cells of the various types of interest in this invention using guanidinium thiocyanate lysis followed by CsCl centrifugation (Chirgwin *et al.*, 1979, *Biochemistry* 18:5294-5299). cDNA is then synthesized from the purified mRNA using, e.g., oligo-dT or random primers. In preferred embodiments, the target polynucleotides are cRNA prepared from purified messenger RNA extracted from cells. As used herein, cRNA is defined here as RNA complementary to the source RNA. The extracted RNAs are amplified using a process in which doubled-stranded cDNAs are synthesized from the RNAs using a primer linked to an RNA polymerase promoter in a direction capable of directing transcription of anti-sense RNA. Anti-sense RNAs or cRNAs are then transcribed from the second strand of the double-stranded cDNAs using an RNA polymerase (see, e.g., U.S. Patent Nos. 5,891,636, 5,716,785; 5,545,522 and 6,132,997; see also, U.S. Patent Application Serial No. 09/411,074, filed October 4, 1999 by Linsley and Schelter and U.S. Provisional Patent Application No. 60/253,641, filed on November 28, 2000, by Ziman *et al.*). Both oligo-dT primers (U.S. Patent Nos. 5,545,522 and 6,132,997) or random primers (U.S. Provisional Patent Application Serial No. 60/253,641, filed on November 28, 2000, by Ziman *et al.*) that contain an RNA polymerase promoter or complement thereof can be used. Preferably, the target polynucleotides are short and/or fragmented polynucleotide molecules which are representative of the original nucleic acid population of the cell.

IN THE CLAIMS:

A marked version of the claims showing the amendments is attached hereto as Exhibit C. Matter that has been deleted from claims 10, 45-46, 86, 157, 182 and 212 is indicated by brackets and matter that has been added is indicated by underlining. A clean version of the pending claims, as amended, is attached hereto as Exhibit D.

Please amend the claims as follows:

Please cancel claims 37-44, 47-85, 91-156 and 184-211, drawn to non-elected subject matter, without prejudice.

Please amend claims 10, 45-46, 86, 157, 182 and 212 to read as follows:

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10. (Amended) The method of claim 1, wherein said measuring is performed by a

method comprising

- A3
- (a) contacting a positionally-addressable array of polynucleotide probes with a sample comprising RNAs or nucleic acids derived therefrom from said cell sample under conditions conducive to hybridization between said probes and said RNAs or nucleic acids, wherein said array comprises a plurality of polynucleotide probes of different nucleotide sequences bound to different regions of a support, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a sequence in a different exon or multiexon of said cell sample; and
 - (b) measuring levels of hybridization between said probes and said RNAs or nucleic acids.

45. (Amended) The method of claim 1 or 10, wherein said organism is a human.

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46. (Amended) The method of claim 1 or 10, wherein said organism is a plant.

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86. (Amended) The method of claim 1 or 10, wherein said cell sample has been subject to a perturbation.

157. (Amended) The method of claim 1, wherein said measuring is performed by a method comprising

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- (a) contacting a positionally-addressable array of polynucleotide probes with a sample comprising RNAs or nucleic acids derived therefrom from said cell sample under conditions conducive to hybridization between said probes and said RNAs or nucleic acids, wherein said array comprises a plurality of polynucleotide probes of different nucleotide sequences bound to different regions of a support, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a sequence in a different exon or multiexon in the genome of an organism from which said cell sample is derived; and
 - (b) measuring levels of hybridization between said probes and said RNAs or

A6
and

nucleic acids.

A7

182. (Amended) The method of claim 157, wherein said expression levels are measured as continuous variables.

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212. (Amended) The method of claim 1 or 10, wherein said organism is a fungus.

Please add the following new claims:

214. (New) The method of claim 2, wherein said measuring is performed by a method comprising

- (a) contacting a positionally-addressable array of polynucleotide probes with a sample comprising RNAs or nucleic acids derived therefrom from said cell sample under conditions conducive to hybridization between said probes and said RNAs or nucleic acids, wherein said array comprises a plurality of polynucleotide probes of different nucleotide sequences bound to different regions of a support, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a sequence in a different exon or multiexon of said cell sample; and
- (b) measuring levels of hybridization between said probes and said RNAs or nucleic acids.

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215. (New) The method of claim 214, wherein said array of polynucleotide probes further comprises control polynucleotide probes comprising sequences complementary and hybridizable to different introns of said plurality of genes in the genome of said organism.

216. (New) The method of claim 214, wherein at least one of said plurality of polynucleotide probes comprises a nucleotide sequence complementary and hybridizable to a sequence spanning the splice junction between different exons in a multiexon.

217. (New) The method of claim 216, wherein said array of polynucleotide probes further comprises control polynucleotide probes comprising sequences complementary and

hybridizable to different introns of said plurality of genes in the genome of said organism.

218. (New) The method of any one of claims 214-217, wherein said plurality of different genes consists of at least 1,000 different genes.

219. (New) The method of any one of claims 214-217, wherein each of said different nucleotide sequences consists of 20 to 100 nucleotides.

220. (New) The method of claim 219, wherein each of said different nucleotide sequences consists of 40 to 80 nucleotides.

221. (New) The method of claim 220, wherein each of said different nucleotide sequences consists of 40 to 70 nucleotides.

222. (New) The method of claim 221, wherein each of said different nucleotide sequences consists of 60 nucleotides.

223. (New) The method of any one of claims 214-217, wherein said organism is a human.

224. (New) The method of any one of claims 214-217, wherein said organism is a plant.

225. (New) The method of any one of claims 214-217, wherein said cell sample has been subjected to a perturbation.

226. (New) The method of claim 3, wherein said measuring is performed by a method comprising

- (a) contacting a positionally-addressable array of polynucleotide probes with a sample comprising RNAs or nucleic acids derived therefrom from said cell

- sample under conditions conducive to hybridization between said probes and said RNAs or nucleic acids, wherein said array comprises a plurality of polynucleotide probes of different nucleotide sequences bound to different regions of a support, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a sequence in a different exon or multiexon of said cell sample; and
- (b) measuring levels of hybridization between said probes and said RNAs or nucleic acids.

227. (New) The method of claim 226, wherein said array of polynucleotide probes further comprises control polynucleotide probes comprising sequences complementary and hybridizable to different introns of said plurality of genes in the genome of said organism.

228. (New) The method of claim 226, wherein at least one of said plurality of polynucleotide probes comprises a nucleotide sequence complementary and hybridizable to a sequence spanning the splice junction between different exons in a multiexon.

229. (New) The method of claim 228, wherein said array of polynucleotide probes further comprises control polynucleotide probes comprising sequences complementary and hybridizable to different introns of said plurality of genes in the genome of said organism.

230. (New) The method of any one of claims 226-229, wherein said plurality of different genes consists of at least 1,000 different genes.

231. (New) The method of any one of claims 226-229, wherein each of said different nucleotide sequences consists of 20 to 100 nucleotides.

232. (New) The method of claim 231, wherein each of said different nucleotide sequences consists of 40 to 80 nucleotides.

233. (New) The method of claim 232, wherein each of said different nucleotide sequences consists of 40 to 70 nucleotides.

234. (New) The method of claim 233, wherein each of said different nucleotide sequences consists of 60 nucleotides.

235. (New) The method of any one of claims 226-229, wherein said organism is a human.

236. (New) The method of any one of claims 226-229, wherein said organism is a plant.

237. (New) The method of any one of claims 226-229, wherein said cell sample has been subjected to a perturbation.

238. (New) The method of claim 2, wherein said measuring is performed by a method comprising

- (a) contacting a positionally-addressable array of polynucleotide probes with a sample comprising RNAs or nucleic acids derived therefrom from said cell sample under conditions conducive to hybridization between said probes and said RNAs or nucleic acids, wherein said array comprises a plurality of polynucleotide probes of different nucleotide sequences bound to different regions of a support, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a sequence in a different exon or multiexon in the genome of an organism from which said cell sample is derived; and
- (b) measuring levels of hybridization between said probes and said RNAs or nucleic acids.

239. (New) The method of claim 238, wherein said array of polynucleotide probes further comprises control polynucleotide probes comprising sequences complementary and hybridizable to different introns of said plurality of genes in the genome of said organism.

240. (New) The method of claim 238, wherein at least one of said plurality of polynucleotide probes comprises a nucleotide sequence complementary and hybridizable to a

sequence spanning the splice junction between different exons in a multiexon.

241. (New) The method of claim 240, wherein said array of polynucleotide probes further comprises control polynucleotide probes comprising sequences complementary and hybridizable to different introns of said plurality of genes in the genome of said organism.

242. (New) The method of any one of claims 238-241, wherein said plurality of different genes consists of at least 1,000 different genes.

243. (New) The method of any one of claims 238-241, wherein each of said different nucleotide sequences consists of 20 to 100 nucleotides.

244. (New) The method of claim 243, wherein each of said different nucleotide sequences consists of 40 to 80 nucleotides.

245. (New) The method of claim 244, wherein each of said different nucleotide sequences consists of 40 to 70 nucleotides.

246. (New) The method of claim 245, wherein each of said different nucleotide sequences consists of 60 nucleotides.

247. (New) The method of any one of claims 238-241, wherein said organism is a human.

248. (New) The method of any one of claims 238-241, wherein said organism is a plant.

249. (New) The method of any one of claims 238-241, wherein said cell sample has been subjected to a perturbation.

250. (New) The method of claim 3, wherein said measuring is performed by a method comprising

- (a) contacting a positionally-addressable array of polynucleotide probes with a sample comprising RNAs or nucleic acids derived therefrom from said cell sample under conditions conducive to hybridization between said probes and said RNAs or nucleic acids, wherein said array comprises a plurality of polynucleotide probes of different nucleotide sequences bound to different regions of a support, each of said different nucleotide sequences comprising a sequence complementary and hybridizable to a sequence in a different exon or multiexon in the genome of an organism from which said cell sample is derived; and
- (b) measuring levels of hybridization between said probes and said RNAs or nucleic acids.

251. (New) The method of claim 250, wherein said array of polynucleotide probes further comprises control polynucleotide probes comprising sequences complementary and hybridizable to different introns of said plurality of genes in the genome of said organism.

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252. (New) The method of claim 250, wherein at least one of said plurality of polynucleotide probes comprises a nucleotide sequence complementary and hybridizable to a sequence spanning the splice junction between different exons in a multiexon.

253. (New) The method of claim 252, wherein said array of polynucleotide probes further comprises control polynucleotide probes comprising sequences complementary and hybridizable to different introns of said plurality of genes in the genome of said organism.

254. (New) The method of any one of claims 250-253, wherein said plurality of different genes consists of at least 1,000 different genes.

255. (New) The method of any one of claims 250-253, wherein each of said different nucleotide sequences consists of 20 to 100 nucleotides.

256. (New) The method of claim 255, wherein each of said different nucleotide sequences consists of 40 to 80 nucleotides.

257. (New) The method of claim 256, wherein each of said different nucleotide sequences consists of 40 to 70 nucleotides.

258. (New) The method of claim 257, wherein each of said different nucleotide sequences consists of 60 nucleotides.

259. (New) The method of any one of claims 250-253, wherein said organism is a human.

260. (New) The method of any one of claims 250-253, wherein said organism is a plant.

261. (New) The method of any one of claims 250-253, wherein said cell sample has been subjected to a perturbation.

262. (New) The method of any one of claims 214-217, 226-229, 238-241 and 250-253, wherein said organism is a fungus.

263. (New) The method of any one of claims 214-217, 226-229, 238-241 and 250-253, wherein said array of polynucleotide probes further comprises one or more sets of successive overlapping probes tiled along the longest variant of an exon.

264. (New) The method of any one of claims 216, 228, 240 and 252, wherein said array of polynucleotide probes further comprises variant junction probes, wherein each of said variant junction probes is specifically hybridizable to a sequence spanning the splice junction between a different variant of an exon and a neighboring exon.

265. (New) The method of claim 86, wherein said perturbation is exposure to a drug.

266. (New) The method of claim 86, wherein said perturbation is a genetic mutation.

267. (New) The method of claim 86, wherein said perturbation comprises mutation of

one or more genes and exposure to a drug.

268. (New) The method of claim 225, wherein said perturbation is exposure to a drug.

269. (New) The method of claim 225, wherein said perturbation is a genetic mutation.

270. (New) The method of claim 225, wherein said perturbation comprises mutation of one or more genes and exposure to a drug.

271. (New) The method of claim 237, wherein said perturbation is exposure to a drug.

272. (New) The method of claim 237, wherein said perturbation is a genetic mutation.

273. (New) The method of claim 237, wherein said perturbation comprises mutation of one or more genes and exposure to a drug.

274. (New) The method of claim 249, wherein said perturbation is exposure to a drug.

275. (New) The method of claim 249, wherein said perturbation is a genetic mutation.

276. (New) The method of claim 249, wherein said perturbation comprises mutation of one or more genes and exposure to a drug.

277. (New) The method of claim 261, wherein said perturbation is exposure to a drug.

278. (New) The method of claim 261, wherein said perturbation is a genetic mutation.

279. (New) The method of claim 261, wherein said perturbation comprises mutation of one or more genes and exposure to a drug.

REMARKS

The specification has been amended to correct a typographical error. The

specification has also been amended to provide the application number of a U. S. provisional patent application. No new matter has been added. A marked version of the paragraphs in the specification which have been amended, with the amendments indicated by bracketing for deletions and underlining for additions, is attached hereto as Exhibit A. A clean version of the paragraphs in the specification, as amended, is attached hereto as Exhibit B.

Claims 37-44, 47-85, 91-156 and 184-211, drawn to non-elected subject matter, have been canceled without prejudice to Applicants' right to pursue the subject matter of these canceled claims in related patent applications. Claims 10, 45-46, 86, 157, 182 and 212 have been amended and new claims 214-279 have been added to more particularly point out and distinctly claim the present invention. Upon entry of the above-made amendment, claims 1-36, 45-46, 86-90, 157-183 and 212-279 will be pending. A marked version of the amended claims showing changes made is attached hereto as Exhibit C. A clean version of the pending claims, as amended, is attached hereto as Exhibit D.

Claims 10 and 157 have been amended to clarify that step (b) of the claimed methods involves measuring *levels of* hybridization between said probes and said RNAs or nucleic acids (emphasis added). Support for the amendments is found in the specification at, e.g., page 7, line 28, through page 8, line 1. Claims 45-46, 86, 182 and 212 have been amended to delete dependency on canceled claims. New claims 214-279 have been added to more particularly point out the invention. Support for the new claims is found in the specification at page 7, line 28, through page 8, line 13; page 8, lines 14-16, lines 22-25, and lines 28-35; page 20, lines 19-33; page 26, lines 3-9; page 35, lines 19-25; page 38, lines 5-8 and lines 9-25; page 43, lines 27-29; page 52, line 26-28; page 55, lines 13-17; page 56, lines 7-27; and page 56, line 30 through page 63, line 12. The above-made amendments are therefore fully supported by the instant application as originally filed and do not constitute new matter.

Claims 1-213 were pending in the application. In the Office Action mailed November 9, 2001, the Examiner has required a restriction to one of the following groups:

Group I: Claims 1-36, 45, 46, 86-90, 157-183 and 212, drawn to a method for analyzing exon expression in a cell sample, classified in class 435, subclass 91.1;

Group II: Claims 37, 39-46, 83, 84, 86-90, 151-156, 182-185, and 212, drawn to

- a method for analyzing exon expression in a cell sample, classified in class 435, subclass 91.1;
- Group III: Claims 38-46 and 212, drawn to a method for determining the presence or absence of alternatively spliced mRNAs for a plurality of genes in a cell sample, classified in class 435, subclass 91.51;
- Group IV: Claims 47, 50-82, 85, 86-90, and 212, drawn to a method for determining the exon expression state of a cell sample, classified in class 435, subclass 91.1;
- Group V: Claims 48 and 49, drawn to a method for determining the exon expression state of a chromosome of an organism in a cell sample, classified in class 435, subclass 91.1;
- Group VI: Claims 91, 92, and 97-127, drawn to an array comprising a positionally-addressable array of polynucleotide probes bound to a support, classified in class 435, subclass 287.2;
- Group VII: Claims 93, 94, and 97-128, drawn to an array comprising a positionally-addressable array of polynucleotide probes bound to a support, classified in class 435, subclass 287.2;
- Group VIII: Claims 95-127, drawn to an array comprising a positionally-addressable array of polynucleotide probes bound to a support, classified in class 435, subclass 287.2;
- Group IX: Claims 129-133, drawn to a set of positionally-addressable array of polynucleotide probes bound to a support, classified in class 536, subclass 23.1;
- Group X: Claims 135 and 136, drawn to a method for preparing an array of

- polynucleotide probes, classified in class 435, subclass 287.2;
- Group XI: Claims 135 and 137, drawn to a method for preparing an array of polynucleotide probes, classified in class 435, subclass 287.2;
- Group XII: Claims 138-145, drawn to a method for determining the relative level of expression of individual exons in a gene, classified in class 435, subclass 91.1;
- Group XIII: Claims 146-150, drawn to a method for detecting alternative splicing between two cell samples of species of an organism, classified in class 435, subclass 91.1;
- Group XIV: Claims 186-196, drawn to a method for determining the effect of a perturbation on RNA splicing pathways in a gene, classified in class 435, subclass 91.51;
- Group XV: Claim 197, drawn to a computer system, classified in class 702, subclass 19;
- Group XVI: Claim 198, drawn to a computer system, classified in class 702, subclass 19;
- Group XVII: Claims 199-201, drawn to a computer system (claims 199 and 200) and a database (claims 200), classified in class 702, subclass 19;
- Group XVIII: Claims 202-204, drawn to a method for selecting polynucleotide probes for preparation of an array for exon profiling, classified in class 435, subclass 91.1; and
- Group XIX: Claims 205-213, drawn to a method for identifying differences in exon or multiexon expression levels, classified in class 435, subclass 91.1

The Examiner contends that the inventions of Groups I-XIX are distinct, each from the other.

At the outset, Applicants respectfully submit that claim 213, dependent on claim 86, properly belongs in Group I. In order to be fully responsive to the Examiner's requirement for a restriction of the instant application, Applicants hereby elect to prosecute the invention of Group I, claims 1-36, 45-46, 86-90, 157-183 and 212-213, drawn to a method for analyzing exon expression in a cell sample, classified in class 435, subclass 91.1.

Entry and consideration of the above-made amendments and remarks are respectfully requested.

Respectfully submitted,

Date: January 9, 2001

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Enclosures